Translation of German Patent D 44 04 625 C2

A method for the inactivation of thermolabile viruses in biological material while maintaining the collagen properties

Description

The subject of the invention is a method in accordance with claims 1 to 5 and the use of thermally treated tissues in accordance with claim 6.

In the manufacture of preserved transplants of human origin for use in medicine, the problems associated with HIV in particular are of great concern.

Serological tests for HIV antibodies do not provide absolute security of HIV absence in the donor; for this reason, additional process steps are required for virus inactivation in the manufacture of preserved transplants.

A chemical treatment by means of hydrogen peroxide is known for this purpose. This method is, however, preferably only suitable for thin, areal material, but is less suited with compact materials (bones, tendons) due to insufficient penetration of the agent.

The disadvantage of insufficient penetration, and thus of insufficient inactivation of viruses in bone materials, should therefore be able to be eliminated by thermal processes, since it is known that HIV inactivation occurs at a temperature of 56°C.

A method for the thermal inactivation of HIV in human bones in a watery medium at a temperature of 80°C and with a treatment time of approximately 10 min. is known, for instance (Unfallchirurg 18/92, 1 - 6 No. 1). This method, however, has the disadvantage that the collagen matrix denaturizes at the temperature used so that the osteoinductive properties which are essential for a bone replacement material are lost or at least greatly reduced. In addition, HIV can admittedly be inactivated at a treatment of 80°C, but not the hepatitis viruses B and C which are only thermolabile at 105°C.

A method is also known from EP 01 41 004 B1 in which bone replacement material is manufactured by thermal sintering of native bone. All organic material is burnt here, including any viruses present, but also the collagen matrix. A bone replacement material produced in this manner no longer has any osteoinductive properties and is thus unsuited for transplant purposes.

An inactivation method is furthermore known which treats bone material with raised steam. This method works e.g. for 10 min. at 121°C and inactivates both HIV and hepatitis viruses, but also simultaneously severely damages the collagen matrix as well as the mineral skeleton itself. A bone transplant autoclaved in this manner therefore has no osteoinductive properties and is thus completely unsuitable as bone replacement material.

Virus inactivation is known from US 4,490,316 by a heat treatment by uses of an organic liquid which, however, relates only to blood plasma

fractions, with the pulverized lyophilisate being heat treated in the form of a suspension.

EP 0 212 041 A1 describes a method for heat inactivation of viruses, but only for the factor VIII preparation, with the heat treatment taking place at a lyophilisate in a dry state in the presence of an inert gas or under a vacuum.

The object of the invention is therefore a method which avoids the disadvantages of the aforesaid chemical or thermal methods.

It was surprisingly found in accordance with the invention that the gentle manufacture allows a biological replacement material for transplant purposes, wherein both an inactivation of thermolabile viruses (such as HIV and hepatitis) takes place and also the required properties of the collagen or collagen-containing tissues are maintained as far as possible.

In accordance with this method, the biological material (e.g. dura mater, fascia lata, fascia temporalis, tendons, vessels, amnion, skin, cartilage, bone as well as other collagen tissue) is subjected to a thermal treatment above the inactivation temperature of viruses, with the heat transfer taking place by means of water-free, or almost water-free (up to a water share of approximately 10%) liquids, vapor or gases. The process of heat transfer can take place at normal pressure or at a reduced pressure or at an elevated pressure or alternately. To achieve the desired heat transfer, mixtures of suitable liquids such as also azeotropic mixtures can also be used.

Suitable liquids are:
methanol, ethanol, propyl alcohol, isopropyl alcohol, isobutanol, acetone, methylethylketone, glycol, glycerin, propandiol, heptane, isoheptane, dimethylsulfoxide, polyethylene glycol silicone oil, 2-3 butanediol.

Suitable gases: N_2, CO_2, noble gases.

The following trial documents the efficacy of the method for the example of the shrinking behavior of a collagen tissue on a thermal treatment in a water-free liquid (e.g. IPA) in comparison with the same treatment in a watery liquid (e.g. water). Dura mater strips of a width of 4 mm and a length of 60 mm are treated in an IPA bath for 20 min. at 80°C:

No shrinking of the tissue occurs. A similar strip treated under the same conditions in a water bath shortens to approximately 50% of its original length within seconds.

It seems quite evident that the structures of the collagen molecule and of the fibrils are irreversibly damaged by the thermal treatment in a watery medium, whereas no deterioration occurs in a non-watery medium.

The intact nature of the collagen tissue is, however, a requirement for suitability as a surgical transplant material. It is plain that e.g. a dura tissue which has suffered irreversible damage as a consequence of shrinking due to a thermal treatment in a watery medium no longer has any native properties.

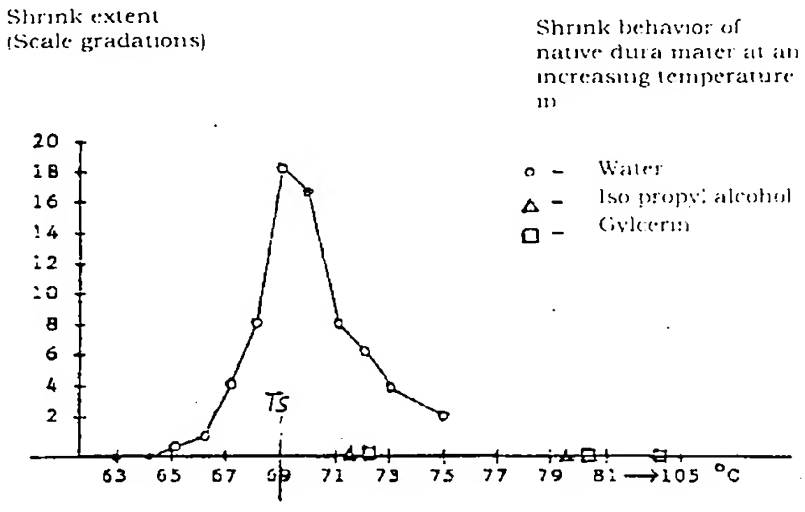
The intact nature of a collagen fiber can be determined by the measurement of the so-called shrink temperature (TS). TS is defined as the temperature at which the collagen fiber has a maximum shrink interval per temperature interval on heating in a watery solution.

For example, a strip of a native dura mater of 4 mm width and 60 mm length shows a TS of 69°C on successive heating in a watery medium (Diagram 1). However, this illustrates the fact that a thermal inactivation of dura or of tendons or of bones is prohibited per se in a water medium at 60°C or above.

In contrast, no shrink occurs on successive heating of native dura to 80°C in a water-free medium, e.g. in IPA, (Diagram 1).

It has surprisingly also been found that even temperatures of e.g. 105°C can be used in the water-free medium without any shrinking occurring (Diagram 1).

Diagram 1



Different properties of tissues treated thermally in watery or non-watery media are also shown in the features of strength and stretch. For instance, native dura strips of comparable thickness with a width of 4 mm show the following relative tensile strength values:

Not thermally treated	100%
Treated for 20 min. at 80°C in an IPA bath	99%
Treated for 20 min. at 105°C in a glycerin bath	96%
Treated for 20 min. at 80°C in a water bath	49%.

The stretch behavior deteriorated accordingly.

The elongation at rupture of the native dura strip not treated thermally and treated thermally in an IPA bath heated to 80°C lies at around 40%, whereas it lies at 150% for the dura strip treated thermally for 20 min. at 80° in water. Such deteriorated properties of transplant tissues with respect to strength and stretching capability are, however, not acceptable for surgical purposes, or only with great limitations.

The same applies analogously to bones treated thermally in a watery medium:

For instance, the pressure load reduces after 10 minutes of autoclaving, e.g. at 121°C in raised steam, to values of approximately 10 – 20% of the starting values.

A thermal treatment in IPA (80°C) or glycerin (105°C) for 20 min. produces a reduction of the values only to approx. 97%.

The method in accordance with the invention will be represented in the examples shown in the following:

1. Dura mater is cleaned in conventional process steps and finally freeze-dried for the purpose of preservation. The water content in the dura now amounts to approximately 5%. For the thermal inactivation, a preserved piece of dura with a size of 4 x 5 cm is put into an IPA bath of 75°C and left in it for 20 min.

Any HIV present in the dura treated in this manner are inactivated and the mechanical properties of the dura show no deterioration (tensile strength: minus 1%) with respect to a dura not thermally treated.

2. A native spongiosa bone of a dimension of 20 x 20 x 20 mm is cleaned in conventional process steps and finally preserved by freeze-drying. The water content of the bone now amounts to approximately 5%. For the thermal inactivation, the preserved piece of bone is treated in a pressure vessel with IPA for 30 min. at 3 bar (approx. 110°C).

Any HIV present in the bone treated in this manner are inactivated and the mechanical properties of the bone show practically no loss of strength with respect to a bone not thermally treated.

3. A native hip bone is treated in a glycerin bath at 110°C for 60 min. for the purpose of thermal inactivation.

Any HIV or hepatitis viruses present in the bone treated in this manner are inactivated and the mechanical properties of the bone show no substantial loss of strength on pressure loading in comparison with a bone not thermally treated.

Subsequently, the hip bone treated with glycerin can be stored by deep freezing e.g. at -40°C.

The method in accordance with the invention can be used for biological tissues in all stages of manufacture, e.g. on femoral heads in a native state or on bone material (in cube or chip form), e.g. during the manufacturing process, or prior to, during or after preservation. The same applies analogously to other biological material made of bone tissue or collagen tissue.

Commercially usable biological replacement material for surgery can be obtained on the basis of different material types and dimensions within the framework of the method in accordance with the invention by modifications in time, temperature and pressure.

Claims

1. A method for the thermal inactivation of thermolabile viruses in collagen or collagen-containing tissues, characterized in that the tissues are exposed in non-watery liquids, vapors or gases to a treatment at an elevated temperature which lies above the respective inactivation temperature of the viruses, while maintaining their transplant properties.

2. A method in accordance with claim 1, wherein the non-watery liquids, vapors or gasses can have a water content of up to 10%.

3. A method in accordance with 1 to 2, wherein the method temperature lies between 50°C and 130°C.

4. A method in accordance with 1 to 3, wherein the temperature treatment can take place at lower pressure, normal pressure or excess pressure or alternately.

5. Use of the tissues thermally treated in accordance with claim 1 to 4 as transplants.

Abstract

A method for the thermal inactivation of thermolabile viruses in collagen or collagen-containing tissues, characterized in that the tissues are exposed to a treatment in non-watery liquids, vapors or gases at an increased temperature which lies above the respective inactivation temperature of the viruses, while maintaining their transplant properties.